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Using Mice to Treat (Wo)men: Mining Genetic Changes in Patient Xenografts to Attack Breast Cancer

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In this issue of *Cell Reports*, Li et al. show that the analysis of genetic changes in patient-derived xenografts can reveal crucial details of tumor evolution, such as the emergence of functional estrogen receptor mutations in endocrine-resistant breast cancer.

The study by Li et al. (2013) published in this issue of *Cell Reports* describes a panel of breast cancer patient-derived xenografts (PDX) generated from aggressive treatment-resistant primary or metastatic breast cancer. The authors use a panoply of approaches, including massively parallel sequencing, RNA sequencing, and reverse phase protein arrays (RPPA), to thoroughly characterize the PDXs as well as perform functional analysis of select candidate mutations. The study is a tour de force, providing PDXs which will endure as invaluable tools and showing that (1) PDXs have relatively stable genomes without a significant accumulation of DNA structural rearrangements but with some enrichment for PDX-unique single-nucleotide variants (SNVs) and (2) there are functional genetic changes in the estrogen receptor (ERα) gene (ESR1) in treatment-resistant disease.

This report is but the latest in a series from a number of laboratories that have recently generated and characterized breast cancer PDX models (e.g., Zhang et al., 2013; Kabos et al., 2012; Ding et al., 2010). A previous genome-wide study by Ding et al. (2010) compared peripheral lymphocytes, primary tumor, and metastasis as well as a PDX model derived from the primary tumor, showing that metastasis and PDX shared enrichment of the same mutations. The current study is an exciting extension of the previous work with additional PDX models and a report of whole-genome sequencing of 17 trios (lymphocytes, tumor, and PDX). Targeted sequencing validated a total of 59,189 SNVs, and variant allele frequency analysis showed that the majority of tumor-PDX pairs had SNV correlation coefficients above 0.65, and the majority of SNVs were maintained at relatively constant levels. Although almost 10% of SNVs were unique to the PDX, the majority (56%) of the nonsilent PDX-specific SNVs (1,056) could not be detected by RNA-seq, suggesting "passenger" roles. However, RNA expression was detected for four of five PDX-unique potentially significant mutations, and 11 of 34 PDX-unique missense mutations, which were predicted to be functionally significant, suggesting at least some enrichment of mutations with potential biological relevance. Intriguingly, there was no enrichment for PDX-specific structural variants, including translocations, large deletions, and inversions.

Many questions arise from these exciting and novel data. What causes the emergence of PDX-unique SNVs? Which changes result from "genetic drift" (i.e., alteration due to random events)? Which PDX-unique mutations are the result of adaption to transplantation into the new microenvironment, and which were present in the original tumor below detectable limits? Is the striking lack of new structural changes in the PDX a reproducible phenomenon, which would suggest fundamental differences in the generation of SNVs and structural variants during tumor evolution? Further studies with additional "trios" using ultra-deep sequencing and approaches that efficiently detect structural variants are necessary in order to answer these questions.

Surely the most exciting finding is the identification of functional ER mutations in ER+ metastatic samples and PDXs. It has generally been a challenge to generate ER+ PDX; however, recent studies (e.g., Kabos et al., 2012; Zhang et al., 2013), have shown that seemingly minor changes in techniques and the use of tumors from advanced disease increase the success rates for the derivation of ER+ PDX. Li et al. (2013) generated eight ER+ PDX models; one from a primary tumor, five from skin metastases, and two from nodal metastases. Four of the PDX models showed estrogen-independent growth consistent with the clinical course of aromatase inhibitor (AI) resistance in patients donating the samples. Sequencing analysis revealed that one of the estrogen-independent tumors harbored an ESR1 Y537S mutation, also recently described by Piccart et al. (data not shown). A Y537N mutation was previously identified and shown to have ligand-independent activities (Zhang et al., 1997; Weis et al., 1996). Breast cancer cells overexpressing Y537N or Y537S mutants responded to the pure antiestrogen fulvestrant, although the response was incomplete. Recent studies showing a role for the Src kinase in phosphorylating Y537,

resulting in coupled target gene activation and ERα turnover, provide an interesting starting point for mechanistic studies (Sun et al., 2012).

In a second estrogen-independent PDX generated from a fulvestrant-resistant tumor, the N terminus of the ESR1 gene had undergone a translocation to the C terminus of YAP1. Given that many translocations do not result in the expression of detectable fusion proteins (Inaki et al., 2011), successful immunoblotting is an important finding. It is also noteworthy that RPPA analysis showed high levels of phosphorylated ERα S118 in this tumor, suggesting that the fusion protein (lacking a functional hormone binding domain) might be activated through posttranslational modifications. Another ESR1 mutation (E380Q) was found in an estrogen-responsive PDX generated from a tamoxifen-responsive tumor. This mutation was not detected in the original skin lesion, suggesting that it might have arisen via adaptation to a low-estrogen environment. Interestingly, E380Q was described more than 20 years ago as the first report of an ER mutant showing ligand-independent activity (Pakdel et al., 1993). Finally, a PDX that regressed after estrogen treatment of the mice showed ESR1 gene amplification and high ERα protein levels. ESR1 amplification was also detected in long-term estrogen-deprived MCF7 cells, suggesting that ESR1 amplification might emerge during estrogen depletion, such as during AI treatment. This finding provides rationale for a biomarker-driven trial in which breast tumors with ESR1 gene amplification are treated with estrogen, a treatment that has been shown to be beneficial in a subset of breast tumors (Ellis et al., 2009).

The study by Li et al. (2013) provides novel evidence of functional ESR1 variants in advanced disease, suggesting that they are selected for during endocrine treatment and, thus, contribute to acquired resistance in a subset of luminal tumors. The field has long wondered why there was such a difference in the prevalence of mutations in ESR1 and androgen receptor (Shafi et al., 2013); in the end, we might see similarities between breast and prostate cancer—molecular changes in the targets of endocrine therapy that lead to distinct clinical behavior and the opportunity for therapeutic targeting.

Furthermore, the study provides evidence that it is possible to recapitulate the spectrum of breast cancer subtypes in PDX with metastatic tissue, and these subtypes are reasonable surrogates for the testing and evaluation of drug effects and mechanisms of resistance. It also shows that surveillance of genomic integrity by comparing the originating primary tumor and PDX can provide information on genetic drift and the potential emergence of mutations that significantly contribute to PDX growth. Given the increasing use of PDX models for therapeutic experiments, which requires the extensive passaging of the tumors, it will be critical to perform systematic analyses in order to determine whether there are additional changes with time.

Finally, this study provides additional illustration of the need to follow Sutton's Law in our efforts to improve outcomes for breast cancer patients—Sutton robbed banks because that was where the money was. We need to evaluate metastatic tumors, because that is where the answers will lie about treatment effects and druggable tumor evolution. A concerted TCGA-like effort for the collection and comprehensive characterization of well-curated metastatic tissue should become a top priority for us as a research community.

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